

REMARKS

Applicants respectfully request reconsideration and allowance of this application in view of the following comments.

Claims 17-21, 23 and 43 were rejected under 35 USC § 103(a) as being obvious over Roth et al. ("Roth"), US 5,545,535, in view of Wan et al. ("Wan"), *J. Immunol. Meth.*, 162: 1-7 (1993), in further view of Cubbage, US 5,582,982. In response, Applicants respectfully submit that the cited combination of references does not make out a *prima facie* case of the obviousness of the instant claims. Therefore, Applicants respectfully request that the Examiner reconsider and withdraw this rejection as well.

According to the Examiner:

"It would have been obvious to one of ordinary skills in the art to use an extra dye such Tryptan blue as taught in Wan to quench extracellular fluorescence in the method of Roth because Roth uses a combination of four fluorescent dyes to stain cells and thus there would be plenty of extracellular fluorescence which would cause non-specific background light, and Tryptan blue can completely quench extracellular fluorescence."

See the second paragraph on page 4 of the Office Action.

Applicants respectfully disagree.

According to Roth, an important aim of the invention described therein is the detection of ***dead cells or membrane-compromised cells***. This can be derived from the

definition of viable/dead cells at column 5, lines 36-53 in combination with, for example, column 22, lines 1-15, where viability of single cells shall be determined. Such determination naturally includes detection of both viable and dead cells. "Viable" cells are defined by Roth at column 5, lines 36-39, as those having "an intact cell membrane." "Dead" cells are defined by Roth at column 5, lines 43-45, as those "where the cell membrane has been *irreversibly disrupted*." Roth also characterizes these "dead" cells as "membrane-compromised cells." *Id.* Also, in the abstract, Roth describes the fluorescent dyes of formula I to IV with respect to their impact on dead bacteria or on cells with comprised plasma membrane integrity.

But exactly because Roth's assays involves detection of these dead and membrane-comprised cells, a person skilled in the art would not, in fact, have been motivated to add Trypan blue or any other masking dye to Roth's assay. Such masking dye would have been expected not only to stain the ambience of the cells and, thus, to reduce the background fluorescence, but would also have been expected *to permeate into the dead cells*, i.e., into the cells "where the cell membrane has been *irreversibly disrupted*" (see, again, column 5, lines 43-45), and also *to quench the signal from these cells*. Such a state of affairs would have rendered Roth's assay unworkable and the results obtained therefrom inaccurate.

The Examiner counters at the bottom of page 6 of the final rejection that:

"[T]he abstract of Roth describes 'A method for analyzing a sample thought to contain bacteria using a total of four dyes which stains all viable and dead cells'. Particularly, dye formula I stains all dead *or* viable cells. Thus, Roth

does not focus on the detection of just dead or membrane-compromised cells but also viable cells. Therefore, *there is a need to quench the extracellular fluorescence of the viable cells* and thus combining Trypan blue would be obvious to one of ordinary skill in the art. [Emphasis added.]”

In response, Applicants believe there are a number of problems with the Examiner’s line of reasoning.

First, no sample of living cells includes viable cells only. Cells constantly die off, thus, Roth’s intended sample would be expected to include both viable and dead cells.

Second, Trypan blue is well known in the art to be *cytotoxic*. Trypan blue acts like a detergent and perforates cell membranes quickly. It is for exactly this reason that Wan teaches removal of Trypan blue after only 1 minute. See, for example, Wan’s abstract (“The dye was removed after 1 min.”) and the first full sentence in the right-hand column on page 3 (“After 1 min, the dye was removed.”) The removal is done quickly to avoid further cell death. Accordingly, using Trypan blue in Roth’s method would have been expected to convert some viable cells into additional dead cells as the assay time wore on.

Some information supporting the cytotoxicity of Trypan blue is attached for the Examiner’s convenience. Since the information supports Applicants’ arguments above, no formal information disclosure statement is required for consideration. *See, MPEP § 609.05(c).*

Roth’s method is intended for use on samples where the tester doesn’t know

whether there are any bacteria therein, let alone whether the bacteria are dead or alive. Then, through the use of one or more of the dyes, the tester can determine whether there are Gram-positive and/or Gram-negative bacteria. Also, the tester can find out whether the bacteria are viable or dead.

A person having ordinary skill in the art, having knowledge of Trypan blue's cytotoxicity, would realize that Trypan blue could not be used in Roth's method. Not only would the Trypan blue permeate the dead and membrane-compromised cells, but, due to its cytotoxicity, over time Trypan blue would create more dead cells, further skewing the results.

In short, modifying Roth to include a masking dye to quench the background fluorescence would have rendered Roth *inoperable for its intended purpose* since such masking dye would also have permeated into the dead cells, which have a disrupted membrane, and quenched the signal from these cells, and, also, killed more cells as the assay time progressed. As set forth in MPEP § 2143.01 (V):

“If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, *there is no suggestion or motivation to make the proposed modification.*”

This legal principle is well-settled and finds ample support in the case law. *See, In re Kramer*, 18 USPQ2d 1415, 1416 (Fed. Cir. 1991) (“[I]t is equally true that if the teachings of a prior art reference would lead one skilled in the art to make a modification which would render another prior art device inoperable, then such a modification would generally not be obvious (italics in original).”) *See, also, In re Gordon*, 221 USPQ 1125,

1127 (Fed. Cir. 1984) (Apparatus, which was required to be turned upside down to meet the terms of the claims, but, by such action would be “rendered inoperable for its intended purpose,” in effect “teaches away from the board’s proposed modification.”)

Respectfully, the Examiner is wrong in asserting that the combination of Roth, Wan and Cubbage would have led to the present invention of the rejected claims. The fact of the matter is that persons skilled in the art would not, in fact, have added a masking dye to Roth’s assay as allegedly taught by Wan, since, as explained above, such addition would have rendered Roth’s assay inoperative for its intended purpose since such masking dye would have been expected to interfere with the signal from Roth’s dead, membrane-disrupted cells, and, also, create more dead cells with time.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw this rejection as well. An early notice that this rejection has been reconsidered and withdrawn is earnestly solicited.

Finally, claim 22 was rejected under 35 USC § 103(a) as being obvious over Roth in view of Wan and further in view of Cubbage and still further in view of Van Aken, US 5,489,537. In response, Applicants point out that this rejection was dependent upon the combination of Roth, Wan and Cubbage making out a *prima facie* case of the obviousness of the broader claims, which Applicants have shown above is not, in fact, the case. Van Aken is relied upon simply to teach Brilliant Black as a fluorescent dye. There is nothing in Van Aken that overcomes the fact that the Examiner’s proposed combination of Roth and Wan would have rendered Roth inoperative for its intended purpose and, therefore, is an improper combination. Consequently, the combination of

Roth, Wan, Cubbage and Van Aken likewise fails to make out a *prima facie* case of the obviousness of claim 22.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw this rejection as well. An early notice that this rejection has also been reconsidered and withdrawn is earnestly solicited.

Applicants believe that the foregoing constitutes a bona fide response to all outstanding objections and rejections.

As this application has been pending an inordinate period of time, i.e., *since September 2001*, Applicants would appreciate receiving an immediate Notice of Allowance. However, should any issue(s) of a minor nature remain, the Examiner is respectfully requested to telephone the undersigned at telephone number (212) 808-0700 so that the issue(s) might be promptly resolved.

Early and favorable action is earnestly solicited.

Respectfully submitted,
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